

The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 146. The invention includes a fragment of a polypeptide comprising SEQ ID NO: 146. The invention includes a diagnostic kit comprising a polypeptide comprising SEQ ID NO: 146, or a fragment thereof. The invention includes a diagnostic kit comprising a polynucleotide sequence encoding SEQ ID NO: 146, or a fragment thereof. The invention includes an immunogenic composition comprising a polypeptide comprising SEQ ID NO: 146, or a fragment thereof. The invention includes an antibody which recognizes a polypeptide comprising SEQ ID NO: 146, or a fragment thereof.

SEQ ID NO: 146 also contains an open reading frame comprising SEQ ID NO: 147. The invention includes a polypeptide comprising SEQ ID NO: 147. SEQ ID NO: 147 is set forth below.

SEQ ID NO: 147

MFIFLLFLTSLTSGSDDLRCITFDVQAPNYTQHTSSMRGVVYPDEIFRSDTLYLTQDFLFP
FYSNVTGFHTINHTFGNVPVIFPKDGIYFAATEKSNVVRGVVFGSTMNNKSQSVIIINNSTN
VVIRACNFELCDNPFFAVSKPMGTQHTMIFDNAFNCTFEYISDAFSLDVSEKSGNFKHL
REFVFNKNDGFLVYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGINITNFRILTAFAQAQDI
WGTSAAAYFVGYLKPTTFMFKYDENGITITDAVDCSQNPLAELKCSVKSFEIDKGIYQTS
NFRVVPBGDVRFPNTITNLCPFGEVFNATKFPVVAWERKKISNCVADSVLYNSTFFST
FKCYGVSAATKLNLCFSNVYADSFVVKGDVVRQIAPGQTVGIADVINYKLPDDFMGCVL
AWNTRNIDATSTGNYNYKYRRLRHGKLRPFERDISNVFPSPDGKCTPPALNCWPLND
YGFYTTTGIGYQPYRVVVLSEFLLNAPATVCGPKLSTDLIKNQCVNFNFGLTGTGVLTP
SSKRQFPFQFGRDVSDFDTSVRDPKTSSELDISPCAFGGVSVITPGTNASSEVAVLYQDV
NCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCI.IGAHEVDTSEYCDIPIGAGICASYH
TVSLIRSTSQKSIVAYTMSLGADSSIAYSNNNTIAIPTNFSISITTEVMPVSMKTSVDCNMY
ICGDSTECANLLQYGSFCTQLNRALSGLAAEQDRNTEVFQAQVKQMYKTPTLKYFGGF
NFSQIIPDLKPKTKRSFIEDLLFNKVTLADAGFMKQYGECLGDINARDLICAKFNGFLT
PPLLTDMDIAAYTAALVSGTATAGWTFGAGAAALQIPFAMQMAYRFNGIGVTONVLYEN
KQIANQFKNKAIQSIESLTTTSTALGLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDI
LSRLDKVAAEVQIDRLITGRILQSLQTYVTQQLIRAAEIRASANAATKMSCEVLGQSKRV
DFCCQGYHILMSFPQAAPHGVVFLHVTYVPSQERNFTTAPACHEGKAAYFPREGVVFVNG
TSWFITQRNFSPQIITDNTFVSGNCDVVIINNNTVYDPLQPELDSFKELDKYFKNHTS
PDVDLGDISGINASVVMNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPPWYVWLGFI
AGLIAVMVTILLCCMTCCKCLKGACSCGSCCKFDEDDSEPVLKGVKLIHYT

The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 147. The invention includes a fragment of a polypeptide comprising SEQ ID NO: 147. The invention includes a diagnostic kit comprising a polypeptide comprising SEQ ID NO: 147, or a fragment thereof. The invention includes a diagnostic kit comprising a polynucleotide sequence encoding SEQ

ID NO: 147, or a fragment thereof. The invention includes an immunogenic composition comprising a polypeptide comprising SEQ ID NO: 147, or a fragment thereof. The invention includes an antibody which recognizes a polypeptide comprising SEQ ID NO: 147, or a fragment thereof. SEQ ID NO: 147 demonstrates functional homology to a coronavirus spike protein.

Predicted transmembrane regions of SEQ ID NO: 147 are identified below.

Predicted Transmembrane helices of SEQ ID NO: 147

The sequence positions in brackets denominate the core region.
Only scores above 500 are considered significant.

Inside to outside helices : 18 found				
	from	to	score	center
1 (1)	16 (16)	959	9	
233 (237)	257 (252)	905	244	
345 (347)	364 (361)	490	354	
345 (354)	369 (369)	420	362	
497 (497)	513 (513)	239	506	
573 (573)	588 (588)	811	580	
645 (648)	666 (663)	302	656	
690 (696)	714 (711)	428	704	
857 (860)	882 (874)	1508	867	
1031 (1031)	1046 (1046)	446	1039	
1199 (1203)	1219 (1217)	2667	1210	

Outside to inside helices : 13 found				
	from	to	score	center
1 (1)	17 (17)	684	10	
222 (222)	240 (237)	238	229	
244 (247)	264 (264)	613	254	
349 (355)	369 (369)	314	362	
496 (496)	511 (511)	488	503	
573 (573)	591 (591)	712	581	
650 (652)	666 (666)	474	659	
674 (679)	702 (696)	190	686	
691 (696)	713 (711)	210	704	
866 (868)	886 (886)	1172	876	
1198 (1201)	1215 (1215)	3221	1208	

SEQ ID NO: 147, the spike protein, is a surface exposed polypeptide. Recombinant expression of a protein can be hindered by hydrophobic transmembrane regions. Accordingly, the invention includes a polypeptide comprising SEQ ID NO: 147 wherein one or more of the hydrophobic regions identified above is removed. The invention further includes a polynucleotide encoding such a polypeptide. The invention includes recombinantly expressing the protein in a host cell.

Further characterization of SEQ ID NO: 147 is set forth below.

PATENT APPLICATION
ATTY REF NO. 20480.004

PSORT — Prediction of Protein Localization Sites

version 6.4 (WWW)

MYSEQ 1255 Residues

Species classification: 4

*** Reasoning Step: 1

Preliminary Calculation of ALOM (threshold: 0.5)

count: 2

Position of the most N-terminal TMS: 496 at i=2

MTOP: membrane topology (Hartmann et al.)

I(middle): 503 Charge difference (C-N): 1.0

McG: Examining signal sequence (McGeoch)

Length of UR: 13

Peak Value of UR: 3.28

Net Charge of CR: 0

Discriminant Score: 8.66

GvH: Examining signal sequence (von Heijne)

Signal Score (-3.5): 5.94

Possible cleavage site: 13

>>> Seems to have a cleavable N-term signal seq.

Amino Acid Composition of Predicted Mature Form:

calculated from 14

ALOM new cnt: 1 ** thrshld changed to -2

Cleavable signal was detected in ALOM?: 0B

ALOM: finding transmembrane regions (Klein et al.)

count: 1 value: -12.26 threshold: -2.0

INTEGRAL Likelihood --12.26 Transmembrane 1202 -1218 (1194 - 1228)

PERIPHERAL Likelihood = 0.16

modified ALOM score: 2.55

>>> Seems to be a Type Ia membrane protein

The cytoplasmic tail is from 1219 to 1255 (37 Residues)

Rule: vesicular pathway

Rule: vesicular pathway

Rule: vesicular pathway

(14) or uncleavable?

Gavel: Examining the boundary of mitochondrial targeting seq.

motif at: 14

Uncleavable? Ipos set to: 24

Discrimination of mitochondrial target seq.:

positive (2.18)

Rule: vesicular pathway

Rule: vesicular pathway

Rule: vesicular pathway

*** Reasoning Step: 2

KDEL Count: 0

Checking apolar signal for intramitochondrial sorting

(Gavel position 24) from: 1 to: 10 Score: 8.0

SKL motif (signal for peroxisomal protein):

pos: 964(1255), count: 1 SRL

SKL score (peroxisome): 0.1

Amino Acid Composition Tendency for Peroxisome: 1.37

AAC not from the N-term., score modified

PATENT APPLICATION
ATTY REF NO. 20480.004

Peroxisomal proteins? Status: notclr
 AAC score (peroxisome): 0.079
 Amino Acid Composition tendency for lysosomal proteins
 score: 0.39 Status: notclr
 GY motif in the tail of typeIa? (lysosomal)
 Checking the amount of Basic Residues (nucleus)
 Checking the 4 residue pattern for Nuclear Targeting
 Checking the 7 residue pattern for Nuclear Targeting
 Checking the Robbins & Dingwall consensus (nucleus)
 Checking the RNA binding motif (nucleus or cytoplasm)
 Nuclear Signal Status: negative (0.00)
 Type Ia is favored for plasma memb. proteins
 Checking the NPXY motif..
 Checking the YXRF motif..
 Checking N myristoylation..

----- Final Results -----

plasma membrane --- Certainty= 0.460(Affirmative) < succ>
 microbody (peroxisome) --- Certainty= 0.171(Affirmative) < succ>
 endoplasmic reticulum (membrane) --- Certainty= 0.100(Affirmative) < succ>
 endoplasmic reticulum (lumen) --- Certainty= 0.100(Affirmative) < succ>

SEQ ID NO: 147 appears to have a N-terminus signaling region, followed by a surface exposed region, followed by a transmembrane region followed by a C-terminus cytoplasmic domain region. Accordingly, the invention includes an immunogenic, surface exposed fragment of SEQ ID NO: 147. Preferably, said fragment comprises an amino acid sequence which does not include the last 50 amino acids of the C-terminus of SEQ ID NO: 147. Preferably, said fragment comprises an amino acid sequence which does not include the last 70 amino acids of the C-terminus of SEQ ID NO: 147. Preferably, said fragment does not include a transdomain region of SEQ ID NO: 147. Preferably, said fragment does not include a C-terminus cytoplasmic domain of SEQ ID NO: 147. Preferably, said fragment does not include a N-terminus signal sequence. Preferably, said fragment does not include amino acids 1 – 10 of the N-terminus of SEQ ID NO: 147. Preferably, said fragment does not include amino acids 1 – 14 of the N-terminus of SEQ ID NO: 147.

The spike protein of coronaviruses may be cleaved into two separate chains into S1 and S2. The chains may remain associated together to form a dimer or a trimer. Accordingly, the invention includes a polypeptide comprising SEQ ID NO: 147 wherein said polypeptide has been cleaved into S1 and S2 domains. The invention further includes a polypeptide comprising SEQ ID NO: 147 wherein amino acids 1 – 10,

preferably amino acids 1 – 14 of the N-terminus are removed and further wherein SEQ ID NO: 147 is cleaved into S1 and S2 domains. Preferably the polypeptide is in the form of a trimer.

Predicted N-glycosylation sites of SEQ ID NO: 147 are identified below:

Prediction of N-glycosylation sites of SEQ ID NO: 147

SeqName	Position	Potential	Jury agreement	NGlyc result
SEQID 147	29 NYTQ	0.7751	(9/9)	+++
SEQID 147	65 NVTG	0.8090	(9/9)	+++
SEQID 147	109 NKSQ	0.6081	(7/9)	+
SEQID 147	119 NSTN	0.7039	(9/9)	++
SEQID 147	158 NCTF	0.5808	(7/9)	+
SEQID 147	227 NITN	0.7518	(9/9)	+++
SEQID 147	269 NGTI	0.6910	(9/9)	++
SEQID 147	318 NITN	0.6414	(9/9)	++
SEQID 147	330 NATK	0.6063	(8/9)	+
SEQID 147	357 NSTF	0.5746	(8/9)	+
SEQID 147	589 NASS	0.5778	(6/9)	+
SEQID 147	602 NCTD	0.6882	(9/9)	++
SEQID 147	699 NFSI	0.5357	(7/9)	+
SEQID 147	783 NFSQ	0.6348	(9/9)	++
SEQID 147	1080 NGTS	0.5806	(7/9)	+
SEQID 147	1116 NNTV	0.5106	(5/9)	+
SEQID 147	1176 NESL	0.6796	(9/9)	++

Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 147 wherein said fragment comprises one or more of the glycosylation sites identified above. The invention further includes a polynucleotide encoding one or more of the fragments identified above. This glycosylation site can be covalently attached to a saccharide. Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 147 wherein said fragment comprises one or more of the glycosylation sites identified above and wherein said polypeptide is glycosylated at one or more of the sites identified above.

Predicted O-glycosylation sites are identified below:

Prediction of O-glycosylation sites

Name	Residue	No.	Potential	Threshold	Assignment
SEQID 147	Thr	698	0.8922	0.7696	T
SEQID 147	Thr	706	0.9598	0.7870	T
SEQID 147	Thr	922	0.9141	0.7338	T
SEQID 147	Ser	36	0.8906	0.7264	S
SEQID 147	Ser	703	0.8412	0.7676	S

The invention includes a polypeptide comprising a fragment of SEQ ID NO: 147 wherein said fragment comprises one or more of the o-glycosylation sites identified above. The invention further includes a polynucleotide encoding one or more of the fragments identified above. The invention further includes a polypeptide comprising a fragment of SEQ ID NO: 147 wherein said fragment comprises one or more of the O-glycosylation sites identified above and further wherein the polypeptide is covalently bonded to a saccharide at one or more of the included glycosylation sites.

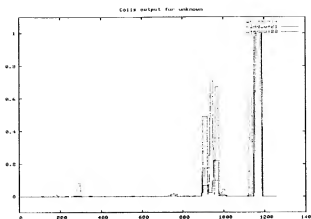
The invention further includes a polypeptide comprising a fragment of SEQ ID NO: 147 wherein said fragment comprises one or more of the N-glycosylation sites identified above and further wherein said fragment comprises one or more of the O-glycosylation sites identified above.

The invention includes a polypeptide comprising a fragment of SEQ ID NO: 147 wherein said fragment does not include one or more of the glycosylation sites identified above. The invention also includes a polynucleotide encoding such a polypeptide.

Predicted phosphorylation sites of SEQ ID NO: 147 are Ser-346, Tyr-195, and Tyr-723. Accordingly, the invention comprises a polypeptide comprising a fragment of SEQ ID NO: 147 wherein said fragment comprises at least ten amino acid residues and wherein said fragment comprises one or more of the amino acids selected from the group consisting of Ser-346, Tyr-195, and Tyr-723. In one embodiment, one or more of the amino acids selected from the group consisting of Ser-346, Tyr-195, and Tyr-723 are phosphorylated.

Predicted coiled coils of SEQ ID NO: 147 are identified below:

Coiled coil Prediction:



Accordingly, the invention comprises a polypeptide sequence comprising a fragment of SEQ ID NO: 147 wherein said fragment includes a coiled region of SEQ ID NO: 147. The invention comprises a polypeptide sequence comprising a fragment of SEQ ID NO: 147, wherein said fragment does not include a coiled region of SEQ ID NO: 147.

The ORF1a and ORF1b sequences of coronaviruses are typically translated as a single ORF1ab polypeptide. Slippage of the ribosome during translation generates an a-1 frameshift. One region of such slippage is illustrated below:

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gggttttacacttagaacaacagctctgtaccgctctgcggaatgtggaaggttatggctgtagttgtga
+1 G F T L R N T V C T V C G M W K G Y G C S C D
+3 G F Y T - K H S L Y R L R N V E R L W L - L -
ccaactccgcgaacccttgatgcagctctgcggatgcatcaacgtttttaaacgggtttgcgggtgaagt
+1 Q L R E P L M Q S A D A S T F L N G F A V - V
+3 P T P R T L D A V C G C I N V F K R V C G V S
gcagcccgctcttacaccgtgcggcacaggcactagactg
+1 Q P V L H R A A Q A L V L
+3 A A R L T P C G T G T S T

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which would generate the following translational slippage:

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ccaactccgcgaacccttgatgcagctctgcggatgcatcaacgtttttaaacgggtttgcgggtgaagt
Q L R E P L M Q S A D A S T F L N R V C G V S

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Accordingly, the invention includes a polypeptide comprising SEQ ID NO: 148.
SEQ ID NO: 148 is set forth below.

SEQ ID NO: 148

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MESLVLGVNETHVQLSLPVLQVRDVLVRGFGDSVEEALSEAREHLKNGTCGLVELEKGLVLPQIEQPYV
FIKRSDALSINHGKVVVELVAEMDGIQYGRSGITLGVLVPHVGETPIAYRNVLRLKNGKNGAGGHSYGI
DLKSYDLGDELGTDPIDIEYQNWNTKHGSGALRELTRLENGGAVTRYVDNNFCGPDGYPFLDCKIDFLAR

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